Improved Survival and Antagonistic Effect of Sodium Fusidate on Tumor Necrosis Factor Alpha in a Neonatal Mouse Model of Endotoxin Shock

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Unlike the antibiotics erythromycin and penicillin G, sodium fusidate (fusidin) pretreatment (80 mg/kg of body weight) increased the survival rate of neonatal BALB/c mice challenged with *Salmonella enteritidis* lipopolysaccharide. Fusidin also significantly reduced the plasma tumor necrosis factor alpha levels. Hence, fusidin may prove useful in the management of bacterial sepsis in humans.

Septic shock is characterized by pathophysiological derangements in the functions of multiple organs (1, 5). Gram-negative sepsis in the newborn continues to be a major cause of mortality (13, 15). It is believed that lipopolysaccharide (LPS), the endotoxin component of the gram-negative bacterial cell wall, is responsible for most of the manifestations of gram-negative sepsis (16, 19). Neonatal septic shock, whether caused by gram-negative or gram-positive bacteria, differs clinically from adult septic shock, giving rise to higher mortality and incidence of serious permanent disabilities (13, 15). Many of the manifestations of shock have been related to high levels of circulating cytokines, particularly tumor necrosis factor alpha (TNF-α) (1, 5, 8, 20).

TNF-α is a potent inflammatory cytokine released mainly by macrophages (2, 5, 8). Treatment with neutralizing anti-TNF-α antibodies attenuates the lethal effects of LPS in adult mice and of gram-negative bacteria in swine and primates (9, 14, 26). We have demonstrated that anti-TNF-α protects neonatal rats and mice against shock caused by LPS or streptococci (18, 25). In the streptococcal shock model, anti-TNF-α prophylaxis was beneficial in neonatal but not adult animals, suggesting a more important pathophysiologic role of TNF-α in neonatal sepsis.

Fusidic acid is an antibiotic with a tetracyclic triterpenoic structure (28). Its chief clinical use is in the treatment of staphylococcal infections (11, 24). Both the acid form and the sodium salt of the drug (fusidin) down-regulate interleukin-1, interleukin-2, and gamma interferon (IFN-γ) production and possess anti-inflammatory and immunosuppressive properties (3, 21–23). In addition, fusidin protects D-galactosamine-sensitized BALB/c mice from staphylococcal enterotoxin B- or LPS-induced lethality and suppresses the in vivo release of TNF-α and IFN-γ (23).

Since neonatal septic shock differs from adult shock both clinically and in the response to anticytokine therapy, the present study was carried out to test the efficacy of fusidin in a neonatal mouse model of LPS-induced shock. Two additional antimicrobial agents, erythromycin and penicillin G, were also tested.

Neonatal ($\leq$24-h-old) BALB/c mice of both sexes were used. Parental mice were obtained from Harlan-Nossan (Milan, Italy). Pups from each litter were randomly assigned to control or experimental groups, marked, and kept with the mother. Before each experiment, LPS from *Salmonella enteritidis* (Sigma Chimica, Milan, Italy) was weighed and diluted in phosphate-buffered saline (PBS; 0.01 M sodium phosphate, 0.15 M sodium chloride, pH 7.2). The neonatal mice were weighed and injected subcutaneously with 25 mg of LPS per kg of body weight. Lethality was assessed at 12-h intervals.

The antibiotics were purchased from Sigma Chimica. Fusidin (sodium fusidate), erythromycin, and the sodium salt of penicillin G were given at the doses indicated below. All antibiotics were given subcutaneously and isovolumetrically (25 μl) 2 h before challenge with LPS. Control animals received an equal amount of vehicle.

In the initial experiments, we evaluated the effects of fusidin, erythromycin, and penicillin G on LPS-induced lethality in neonatal mice. Figure 1 shows that treatment 2 h before challenge with 80 mg of fusidin per kg significantly increased survival 24 and 48 h after challenge. This effect, however, was no longer significant at 72 and 96 h, indicating a lack of permanent protection by a single injection of the drug before LPS challenge. This dose of fusidin was selected on the basis our previous study in adult shock models (23). Higher doses resulted in inflammatory reactions at the injection site and decreased suckling activity for about 2 days after injection. There was no effect on survival when fusidin was given at the time of challenge with LPS or at later times (data not shown). Unlike fusidin, neither erythromycin (33 mg/kg) nor penicillin G (130 mg/kg) reduced lethality (Fig. 1). The slight enhancement in survival afforded by erythromycin was not statistically significant. Increasing the dose of erythromycin and penicillin to 70 and 240 mg/kg, respectively, was also ineffective (data not shown).

TNF plays a central role in most if not all LPS models of lethality (5, 27), and since fusidin modulates TNF-α production in adult rodents (6, 23), the effect of the drug on circulating TNF-α levels in the neonatal mice was also investigated. Groups of six animals were killed by decapitation under ether anesthesia at different times after challenge with LPS. Mixed venous-arterial blood was collected in heparinized containers

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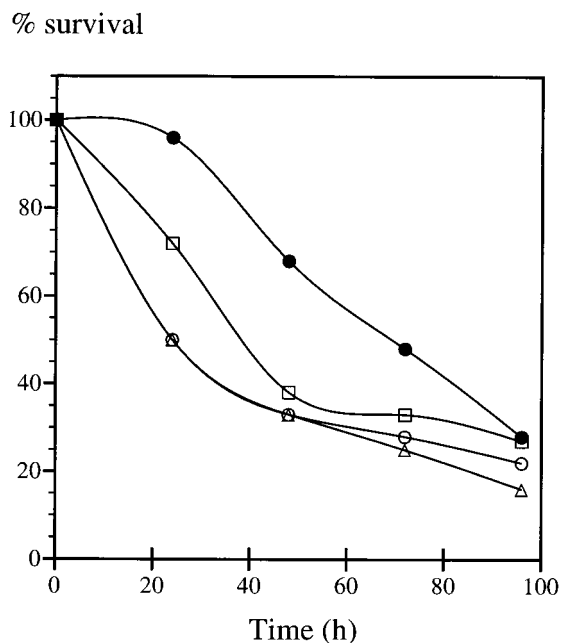
% survival

FIG. 1. Effects of pretreatment (2 h) with different antibiotics on lethality induced by *S. enteritidis* LPS. ○, control (*n* = 36); ●, fusidin (80 mg/kg; *n* = 25; *P* < 0.05, log rank Mantel-Cox test); △, penicillin G (130 mg/kg; *n* = 12; *P* = 0.79); □, erythromycin (33 mg/kg; *n* = 18; *P* = 0.45).
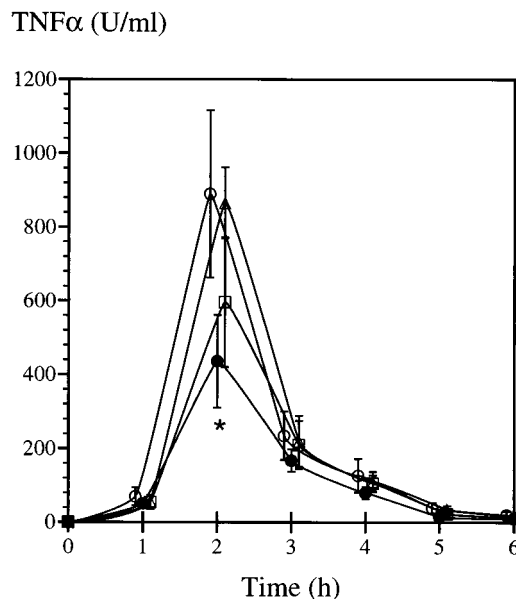
TNFα (U/ml)

FIG. 2. Plasma TNF-α levels in neonatal mice treated with different antibiotics 2 h before challenge with LPS. The data are shown as mean values (± standard deviations). Symbols are as in Fig. 1. Each group consisted of six mice, with three determinations per group (see text). *, significantly different from control (*P* < 0.05, one-way analysis of variance and the Student-Newman-Keuls test).

and centrifuged. Plasma samples from two animals were pooled for TNF-α measurement. TNF-α activity was expressed in units per milliliter, 1 U being defined as the amount of cytokine causing 50% lysis of the WEHI 164 cell clone 13 (10). To rule out interference with the bioassay, preliminary experiments showed that fusidin (100 μg/ml) failed to influence WEHI 164 cell lysis induced by murine recombinant TNF-α (specific activity, 20 U/ng; Genzyme, Cinisello Balsamo, Italy). Seven serial twofold dilutions (final concentrations, 1:20 to 1:1,280) were tested in duplicate. The assay was calibrated with murine recombinant TNF-α (Genzyme) as a standard. Since sera were diluted 20 times before assay, the actual lower limit of detection was 20 U of TNF-α per ml. To calculate mean values, results below the detection level (20 U/ml) were assigned a theoretical value of 10 U/ml.

As shown in Fig. 2, plasma TNF-α bioactivity was not detected in untreated or zero time controls receiving LPS. In the PBS-pretreated animals, the TNF-α values peaked at 2 h and returned to baseline values at 5 to 6 h after LPS injection. Pretreatment with fusidin, but not erythromycin and penicillin G, significantly decreased the TNF-α peak values (*P* < 0.05).

Since TNF-α was previously shown to play an important pathophysiological role in a similar shock model (18), it is possible that the beneficial effect of fusidin on survival is due, at least in part, to the drug's ability to decrease TNF-α production in vivo. This assumption is in agreement with results of a previous study, in which fusidin protected adult mice against both LPS- and staphylococcal enterotoxin B-induced shock (23). In that study, lethality was completely prevented, whereas the present study reveals only a significant increase in survival. This difference can be accounted for by differences between adults and neonates in their cytokine responses to LPS. For example, IFN-γ, a cytokine whose production in vitro and in vivo is markedly down-regulated by fusidin (3, 21), is likely to play a more important role in adult than in neonatal rodent

shock models (4, 12). Thus, 24-h-old mice produce little circulating IFN-γ in response to LPS compared with adult animals (7). Moreover, neonatal mice are less responsive than adult mice to treatment with TNF-α and eicosanoid inhibitors, possibly resulting from LPS-induced derangement in carbohydrate metabolism (17, 29).

The efficacy of fusidin in the treatment of staphylococcal infections is well documented (28). Staphylococci are being increasingly reported as an important cause of invasive infections in hospitalized patients, and gram-positive bacteria are now a more frequent cause of septic shock than gram-negative bacteria in many hospitals (27). Because fusidin may also down-regulate the production of inflammatory cytokines during septic conditions, this antibiotic may prove particularly useful in the treatment of patients with sepsis caused by gram-positive bacteria. However, since fusidin exhibited significant effects in the neonatal mouse model only when given prophylactically, the potentials of fusidin therapy in human sepsis conditions remain to be shown.

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